FORM PTO-1390 (Modified) (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
196737US0PCT	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	09/622915

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCTJP99/01146	10 March 1999	10 March 1998

TITLE OF INVENTION
FULLERENE DERIVATIVE

APPLICANT(S) FOR DO/EO/US
Eiichi NAKAMURA, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
    A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☐ Certificate of Mailing by Express Mail
19. ☒ Other items or information:

Request for Consideration of Documents Cited in International Search Report
Notice of Priority
PCT/IB/304
PCT/IB/308
Drawing (1 sheet)

533 Rec'd PCT/PTO 07 SEP 2000

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/622915	PCTJP99/01146	196737US0PCT

20. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☒	Search Report has been prepared by the EPO or JPO	**$840.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482)	**$670.00**
☐	No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	**$760.00**
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO	**$970.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	**$96.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$840.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	21 - 20 =	1	x $18.00	**$18.00**	
Independent claims	7 - 3 =	4	x $78.00	**$312.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,300.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	**$0.00**	
SUBTOTAL =				**$1,300.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
TOTAL NATIONAL FEE =				**$1,300.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED =				**$1,300.00**	
				Amount to be: refunded	$
				charged	$

☒ A check in the amount of **$1,300.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☐ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

NEUSTADT, P.C.

22850

Surinder Sachar
Registration No. 34,423

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

Sept 7 2000
DATE

SCANNED, #7

SPECIFICATION

FULLERENE DERIVATIVE

TECHNICAL FIELD

The present invention relates to a fullerene derivative which has DNA compacting activity and is useful as a DNA compaction reagent, among other uses, and is applicable, for example, in the pharmaceutical industry.

BACKGROUND ART

DNA compaction in a protein-DNA complex such as the arrangement of DNAs on a chromosome is a very important subject of biochemical research. Compaction by organic micromolecules and inorganic ions is also an important subject of research relevant to transfection [e.g. Yoshikawa, Y. et al., FEBS Letters, 1996, vol. 396, 71-76; Behr, J-P, Acc. Chem. Res., 1993, vol. 26, 274-278; etc.].

The present invention has for its object to provide a novel means for DNA compaction.

DISCLOSURE OF INVENTION

Thus far has been provided a technology for use-tailored modification of fullerene, and a variety

of fullerene derivatives have been synthesized [e.g. Friedman, S. H. et al. J. Am. Chem. Soc., 1993, vol. 115, 6506-6509; Yamago, S. et al., J. Am. Chem. Soc., 1994, vol. 116, 1123; Taki, M. et al., J. Am. Chem. Soc., 1997, vol. 119, 926; An, Y. Z. et al., Tetrahedron, 1996, vol. 52, 5179-5189; Nakamura, E. et al., Bull. Chem. Soc. Jpn., 1996, vol. 69, 2143-2151; Yamago, S. et al., Chemistry Letters, 1996, 395-396; Murata, Y. et al., The 2nd International Forum on Chemistry of Functional Organic Chemicals (IFOC-2), 1997, P-31, Tokyo, Japan, etc.].

The inventors of the present invention discovered that, among such fullerene derivatives, fullerene derivatives having 1 to 4, nitrogen-containing hydrophilic side chain(s), inclusive of salts thereof, are amphiphilic and have exceptionally high DNA-compacting activity and have accordingly developed the present invention.

1. Structure of the fullerene derivative of the invention

The fullerene derivative of the present invention is a "fullerene derivative having 1 to 4, nitrogen-containing hydrophilic side chain(s)". This fullerene

derivative includes not only novel compounds but also known compounds.

The DNA-compacting activity of the fullerene derivative of the present invention is the result of an interplay of the size and hydrophobicity of fullerene and the affinity of the nitrogen-containing hydrophilic side chain(s) of the derivative for the phosphate group. It is supposed that the interaction between fullerene and the hydrophobic moieties of a DNA (e.g. major grooves of the DNA) and the interaction between the nitrogen-containing hydrophilic side chain(s) and the phosphate group of the DNA causes the DNA unimolecule to be bent and folded, and that the hydrophobic moieties of a large number of such folded DNA unimolecules coalesce to cause said compaction.

Therefore, the molecular design of a fullerene derivative may be made liberally by one skilled in the art with the above mechanism taken into consideration. The DNA-compacting activity of the fullerene derivative synthesized accordingly can be evaluated by electrophoresis of a mixed solution of the fullerene derivative and a DNA (e.g. plasmid DNA) and measuring the amount of DNA. Moreover, since this compacting activity of the fullerene derivative is closely associated with the high binding affinity of the

derivative for DNA, a screening can be made by an ethidium bromide displacement assay using calf thymus DNA.

While the fullerene derivative may be used in the form of a salt, the salt is preferably a conventionally nontoxic salt, particularly a pharmaceutically acceptable salt. More particularly, the salt includes inorganic acid salts (e.g. hydrochloride, hydrobromide, sulfate, phosphate, etc.), organic carboxylic acid or sulfonic acid salts (e.g. formate, acetate, trifluoroacetate, maleate, tartrate, fumarate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.), and salts with basic or acidic amino acids (e.g. arginine, aspartic acid, glutamic acid, etc.).

The fullerene derivative may occur as various isomers owing to the presence of asymmetric carbon and molecular asymmetry and any and all of them are subsumed under the concept of fullerene derivative according to the present invention.

The "fullerene" of the fullerene derivative of the present invention is not restricted to [60]fullerene but includes higher-order fullerenes (e.g. [70]fullerene etc.).

The preferred "nitrogen-containing hydrophilic side chain" includes "a hydrocarbon group which has 1

or 2 straight-chain or branched-chain substituent group(s) each comprising 1 to 10 nitrogen atom(s) and 2 to 30 carbon atoms, and is configured to be bonded to 1 or 2 of the 2 to 8 sp^3 carbon atoms present on the fullerene core". The more preferred is "a hydrocarbon group which has 1 or 2 straight-chain or branched-chain substituent group(s) each comprising 2 to 8 nitrogen atoms and 2 to 20 carbon atoms, and is configured to be bonded to two of the 2 to 8 sp^3 carbon atoms present on the fullerene core".

The amino group in said "nitrogen-containing hydrophilic side chain" may be primary, secondary or tertiary and may form a nitrogen-containing heterocyclic group [such as 3 to 8 (preferably 5 or 6)-membered unsaturated hetero-monocyclic groups containing 1 to 4 nitrogen atom(s) (e.g. pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, dihydropyridyl, pyrimidyl, pyrazinyl, pyridazinyl, triazolyl, tetrazolyl, etc.); and unsaturated fused heterocyclic groups containing 1 to 4 nitrogen atom(s) (e.g. indolyl, isoindolyl, indolidinyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl, acridinyl, etc.)]. Furthermore, it may optionally be substituted by lower alkyl or the like.

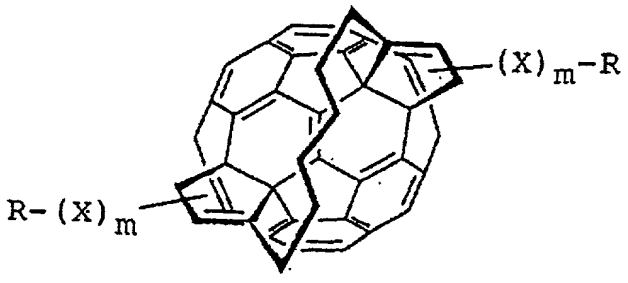
The "nitrogen-containing hydrophilic side chain"

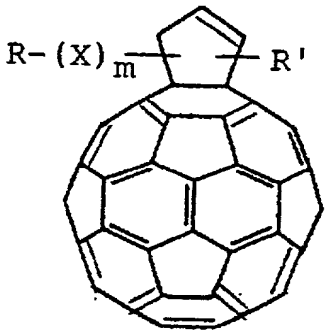
mentioned above may have other hetero atoms, such as oxygen, sulfur, etc., as its constituent atoms and/or substituents.

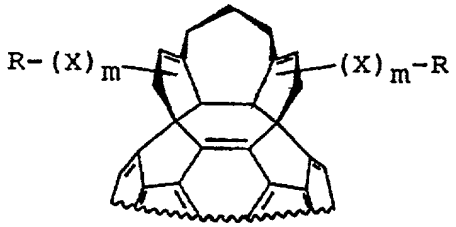
Furthermore, when two or more "nitrogen-containing side chains" are present, there may be a cross-linking alkylene moiety bridging such nitrogen-containing hydrophilic side chains.

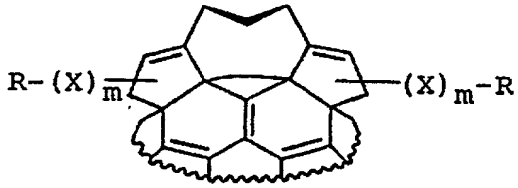
The "hydrocarbon group" of the "nitrogen-containing hydrophilic side chain" includes straight-chain, branched-chain, or cyclic hydrocarbon groups, whether saturated or unsaturated, and is preferably a hydrocarbon group of 1 to 20 carbon atom(s) (more preferably of 1 to 15 carbon atom(s)).

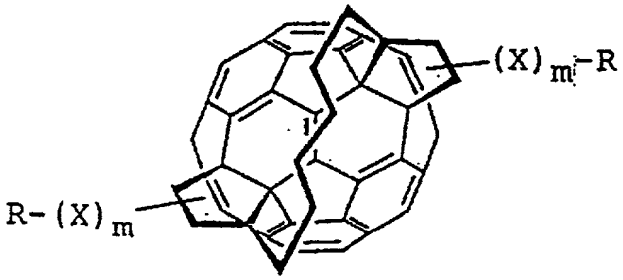
The specific structure of said "nitrogen-containing hydrophilic side chain" includes but is not limited to the following (the fullerene core is also shown).

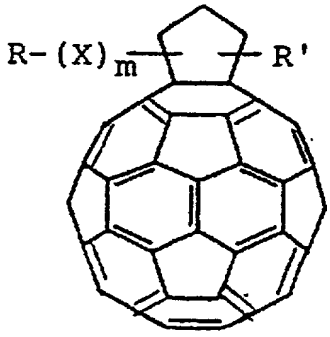


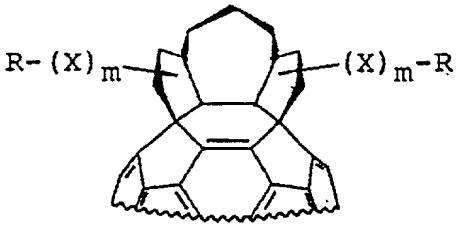


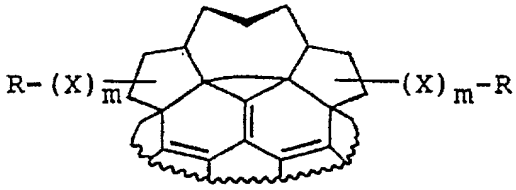

R-(X)m
(X)m-R

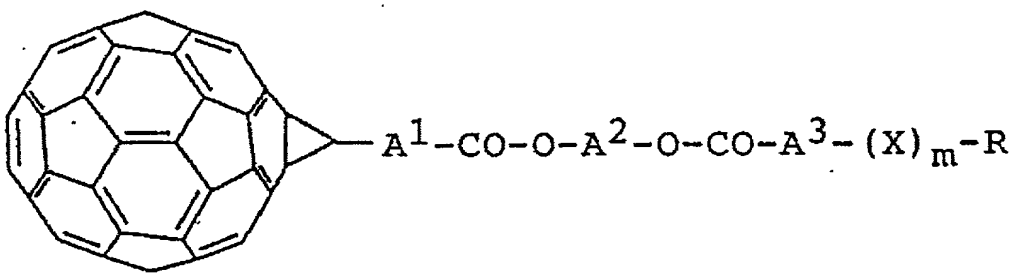

R-(X)m
(X)m-R

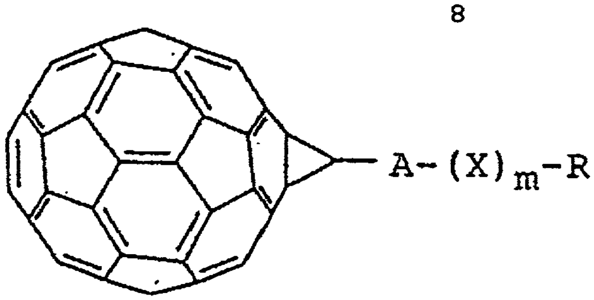

(X)m-R
R-(X)m

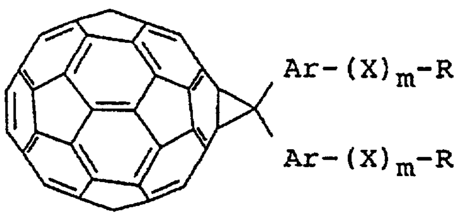

R-(X)m
R'

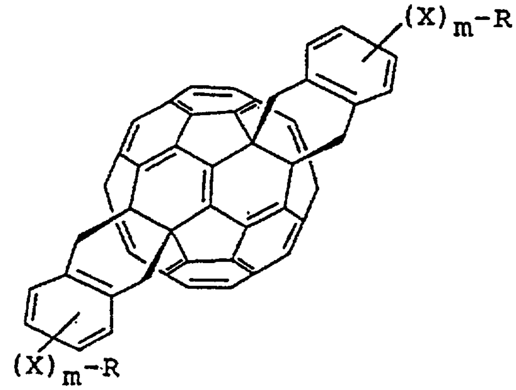

R-(X)m
(X)m-R

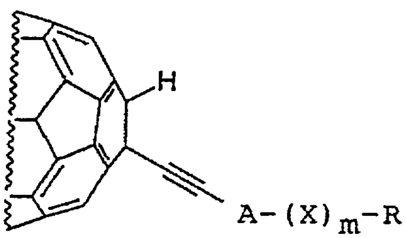

R-(X)m
(X)m-R

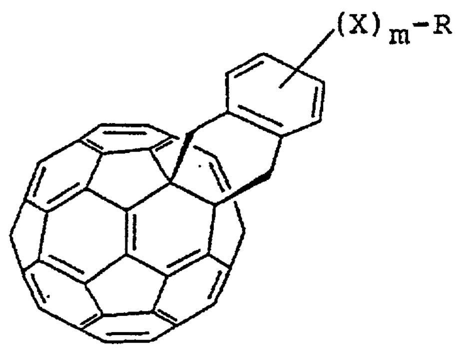

A1-CO-O-A2-O-CO-A3-(X)m-R

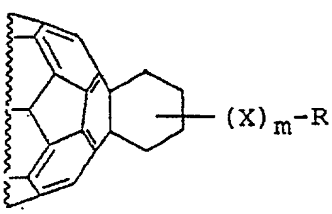


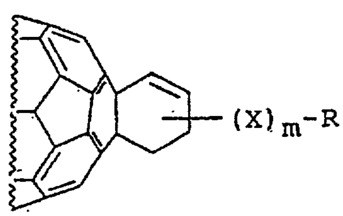


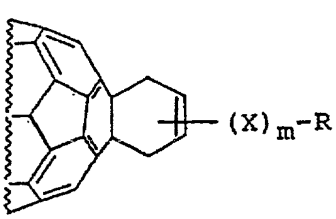


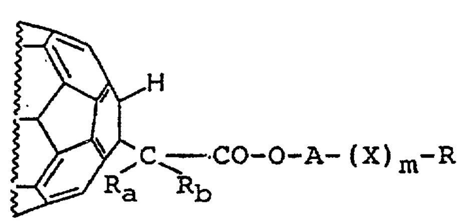




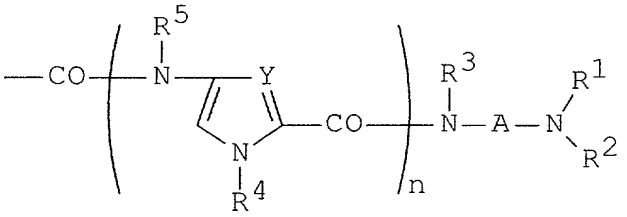






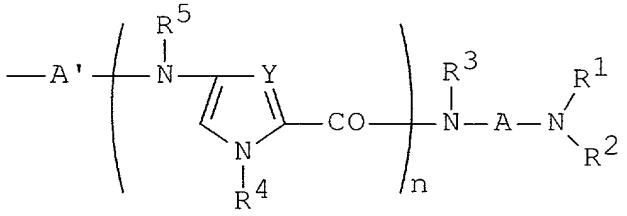


In the above formulas, Rs may be the same or different and each represents a straight-chain or branched-chain acyl group comprising 1 to 10 nitrogen atom(s) and 2 to 30 carbon atoms [more preferably [N-(N,N-di(lower)alkylamino)(lower)alkyl-N-(lower)alkyl]amino(lower)alkanoyl groups, [N-(N-(lower)alkylamino)(lower)alkyl-N-(lower)alkyl]-amino(lower)alkanoyl groups, [N-pyrrolyl(lower)-alkyl-N-(lower)alkyl]amino(lower)alkanoyl groups, [N-(N,N-di(lower)alkylamino)(higher)alkyl-N-(lower)alkyl]amino(lower)alkanoyl groups, [N-(N-(lower)alkylamino)(lower)alkyl-N-(lower)alkyl]-amino(higher)alkanoyl groups, [N-pyrrolyl(higher)-alkyl-N-(lower)alkyl]amino(higher)alkanoyl groups; groups of the formula:



(wherein R^1, R^2, R^3, R^4 and R^5 may each be the same or different over its occurrences and represents hydrogen or a lower alkyl group; A represents an alkylene group; Y represents CH or N, and n represents an integer of 1 to 4)]; straight-chain or branched-chain C_{2-30} alkyl

groups comprising 1 to 10 nitrogen atom(s) and 2 to 30 carbon atoms [more preferably [N-(N,N-di(lower)alkylamino)(lower)alkyl-N-(lower)alkyl]amino(lower)alkyl groups, [N-(N-(lower)alkylamino)(lower)alkyl-N-(lower)alkyl]amino(lower)alkyl groups, [N-pyrrolyl(lower)alkyl-N-(lower)alkyl]amino(lower)-alkyl groups, [N-(N,N-di(lower)alkylamino)(higher)-alkyl-N-(lower)alkyl]amino(lower)alkyl groups, [N-(N-(lower)alkylamino)(lower)alkyl-N-(lower)alkyl]-amino(higher)alkyl groups, [N-pyrrolyl(higher)-alkyl-N-(lower)alkyl]amino(higher)alkyl groups; or groups of the formula:



(wherein R^1, R^2, R^3, R^4, R^5, A, Y and n are respectively as defined above; A' represents an alkylene group)].

Ar represents an aryl group (e.g. phenyl, naphthyl, anthryl, etc.);

R' represents hydrogen or a lower alkyl group;

Ra and Rb may be the same or different and each represents hydrogen or a lower alkyl group, or Ra and

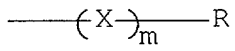
Rb may, jointly and taken together with the carbon atom to which they are joined, represent a 3 to 6-membered cycloalkyl group.

A, A^1, A^2 and A^3 may be the same or different and each represents an alkylene group;

X represents -O-, -N- or -S-; and

m represents an integer of 0 or 1.

It should, however, be understood that the various "nitrogen-containing hydrophilic side chains" mentioned above are mere examples and, as the structure interposed between "the fullerene core" and "the group of the formula



the various known structures other than those illustrated above may also be selectively used.

The "lower alkyl group" or "lower alkyl moiety" in the context of the present invention includes straight-chain or branched-chain groups each containing 1 to 6 carbon atom(s), such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, tert-pentyl, neopentyl, hexyl, isohexyl, etc.

The "alkylene group" includes straight-chain or branched-chain groups containing 1 to 10 carbon atom(s), such as methylene, ethylene, trimethylene, 2-

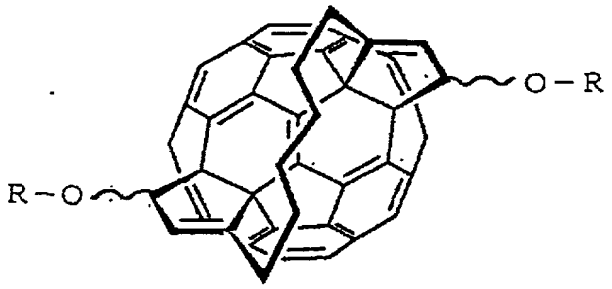
methyltrimethylene, tetramethylene, ethylethylene, pentamethylene, 3-methylpentamethylene, hexamethylene, 2-ethyltetramethylene, heptamethylene, octamethylene, nonamethylene, decamethylene, etc.

The "higher alkyl group" or "higher alkyl moiety" includes straight-chain or branched-chain groups each containing 7 to 20 carbon atoms, such as heptyl, octyl, 2-ethylhexyl, nonyl, decyl, 3,7-dimethyloctyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, 3-methyl-10-ethyldodecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, etc.

The number of "nitrogen-containing hydrophilic side chains" on the fullerene core is preferably 1 to 4, and the presence of one or two such side chains is particularly preferred. When only one side chain is to be used, it is preferable to select a side chain containing a relatively large number of nitrogen atoms (preferably 4 or more N atoms). Particularly preferred is a polypyrrole which has a comparatively high binding affinity. When two side chains are to be involved, the use of alkylpolyamines containing 2 to 8 nitrogen atoms, which are comparatively less hydrophilic, is preferred.

Among the various fullerene derivatives described above, suitable derivatives can be judiciously selected in consideration of the ease of synthesis and the

binding affinity for DNA, among other factors, but based on the information so far available, derivatives of the following general formula (I), inclusive of salts thereof, can be mentioned as preferred examples.



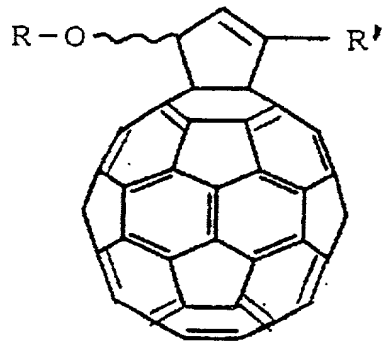
[wherein the two Rs may be the same or different and each represents a straight-chain or branched-chain acyl group comprising 1 to 10 nitrogen atom(s) and 2 to 30 carbon atoms or hydrogen (provided, however, that the two Rs do not concurrently represent hydrogen)].

As the more preferred examples of the fullerene derivative, there can be mentioned derivatives of general formula (I) wherein the two Rs are the same or different and each represents a straight-chain or branched-chain acyl group comprising 2 to 8 nitrogen atoms and 2 to 30 carbon atoms as its constituent atoms.

As the still more preferred examples of the fullerene derivative, there can be mentioned derivatives of general formula (I) wherein the two Rs are the same or different and each represents a

straight-chain or branched-chain acyl group comprising 2 to 8 nitrogen atoms and 2 to 20 carbon atoms as its constituent atoms.

Furthermore, fullerene derivatives of the following general formula (II):



[wherein R represents a straight-chain or branched-chain acyl group comprising 1 to 10 nitrogen atom(s) and 2 to 30 carbon atoms and R′ represents hydrogen or a lower alkyl group] inclusive of salts thereof can also be mentioned as preferred examples of the fullerene derivative.

As the more preferred examples of the fullerene derivative, there can be mentioned derivatives of general formula (II) wherein R represents a straight-chain or branched-chain acyl group comprising 2 to 8 nitrogen atoms and 2 to 30 carbon atoms.

The still more preferred examples of the fullerene derivative are derivatives of general formula (II) wherein R represents a straight-chain or branched-chain

acyl group comprising 2 to 8 nitrogen atoms and 2 to 20 carbon atoms.

2. Method of producing the fullerene derivative of the invention

The above-mentioned fullerene derivatives and salts of the present invention can be synthesized by the processes known to those skilled in the art, either as such or as appropriately modified, according to the respective structures desired [cf. the literature cited above or below].

The method of producing the fullerene derivative of the invention is now described in further detail, taking fullerene derivatives having one or two nitrogen-containing hydrophilic side chains" as examples.

Process A (one side chain)

The organofullerene (methanofullerene, propanofullerene) obtainable by carrying out the known reaction of the vinylcarbene species thermally generated from a cyclopropenone acetal with fullerene (Literature 1; Tokuyama, H.; Isobe, H.; Nakamura, E., Bull. Chem. Soc. Jpn. 1995, vol. 68, 935-941) is subjected to a functional group transformation reaction to introduce a nitrogen-containing side chain.

Methanofullerene and propanofullerene are respectively converted to hydroxy-containing organofullerenes, by the procedure which comprises adding water after the reaction of said vinylcarbene species to hydrolyze the ketene acetal in the case of methanofullerene and by the procedure which comprises hydrolytic removal of the acetal with the aid of the sulfuric acid catalyst in water/tetrahydrofuran/chlorobenzene and subsequent reduction with diisobutylaluminum hydride. The objective fullerene derivative can be synthesized by subjecting the thus-generated hydroxyl group to the following functional group transformation.

1. Known reaction (Literature 2, Nakamura, E.; Tokuyama, H.; Yamago, S.; Shiraki, T.; Sugiura, Y., Bull. Chem. Soc. Jpn. 1996, vol. 69, 2143-2151). By the coupling reaction of succinic anhydride and a hydroxy-containing organofullerene, the carboxylic acid derivative is prepared. This carboxylic acid and an amine compound having a primary or secondary amino function are subjected to coupling reaction to give the objective product.

As the amine compound mentioned just above, a polypyrrole derivative analogous to the netropsin derivative described in Literature 2, a polyamine such as an alkylspermidine or the like, and even acridine

or the like which is intercalatable into the DNA base pair can be employed, for instance.

2. An α-haloacid halide is coupled to a hydroxyl group-containing organofullerene (Literature 3: Boutorine, A. S.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Helene, C., Angew. Chem., Int. Ed. Engl., 1994, vol. 33, 2462-2465; Literature 4: An, Y. Z.; Chen, C. H. B.; Anderson, J. L. Sigman; D. S. Foote, C. S.; Rubin, Y., Tetrahedron, 1996, vol. 52, 5179-5189) to give an α-halocarbonyl compound. The objective product can be obtained by coupling this halide to an amine compound having a primary or secondary amino function.

As this amine compound, the specific amine compounds mentioned hereinbefore can be employed.

These procedures can be applied to the known hydroxy-containing fullerene derivatives (Literature 4 cited above; Literature 5: Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura, Y., J. Am. Chem. Soc., 1993, vol. 115, 7918-7919) in common.

Process B (2 side chains)

A fullerene derivative having more potent DNA-compacting activity can be synthesized from an organofullerene (bispropanofullerene) obtained by carrying out the known reaction of a biscyclopropenone

acetal with a fullerene (Literature 6: Isobe, H.; Tokuyama, H.; Sawamura, M.; Nakamura, E., J. Org. Chem., 1997, vol. 62, 5034-5041) in conjunction with the procedure of said Process A by subjecting it to functional group transformation to thereby introduce hydrophilic residues. The functional group transformation of bispropanofullerene is carried out, just as mentioned above, by the hydrolytic removal of the acetal in the presence of the sulfuric acid catalyst in water/tetrahydrofuran/chlorobenzene and subsequent reduction with diisobutylaluminum hydride, whereby the bispropanofullerene is converted to an organofullerene having a couple of hydroxyl groups. In this procedure, a mixture of 8 different isomers inclusive of diastereomers is obtained, and the objective fullerene derivative can be synthesized by subjecting this mixture to the same functional group transformation as above.

1. Succinic anhydride is coupled to a hydroxy-containing organofullerene to give the carboxylic acid derivative. This carboxylic acid is coupled to an amine compound having a primary or secondary amino function to obtain the desired product.

As the amine compound, the same specific compounds as mentioned above can be employed.

2. An α-haloacid halide is coupled to an organofullerene having two hydroxyl groups to give the corresponding α-halocarbonyl compound. As the α-haloacid halide for use in this procedure, α-bromoacetyl bromide is known. As the halide, both the chloride and the bromide can be utilized and even the compound bearing a substituent in the α-position can also be used. Regarding the organofullerene, a report is available on the derivatives having the C_2 symmetry (Literature 7: Isobe, H.; Sawamura, M.; Nakamura, E., 13th Fullerene Symposium, 1997, 2-20, Nagano, Japan) but the equivalent or higher activity may be obtained by using organofullerenes of other symmetries as described in the above-cited Literature 6 or a mixture of isomers obtainable by said reduction with diisobutylaluminum hydride. The coupling of the resulting halide to an amine compound having a primary or secondary amino function, such as the compound mentioned above, gives the objective product.

The above amine compound includes the species mentioned hereinbefore.

The above procedures can be applied to any known fullerene derivatives having a plurality of hydroxyl groups (Literature 8: Taki, M.; Sugita, S.; Nakamura, Y.; Kasashima, E.; Yashima, E.; Okamoto, Y.; Nishimura,

J., J. Am. Chem. Soc., 1997, vol. 119, 926) as well.

Those skilled in the art should be able to produce fullerene derivatives having desired structures in accordance with the invention by referring to the disclosures in the literature cited in this specification, known technologies, and the specific disclosures concerning Processes A and B, or working examples which are presented hereinafter.

3. Modes of use of the fullerene derivative of the invention

The fullerene derivative of the present invention is an amphiphilic compound having excellent DNA-compacting activity. Permitting the fullerene derivative of the invention to act on DNA leads to the result that the derivative binds the DNA and the unimolecular DNA is bent and folded by the interbinding force acting between the intramolecular double strands. Furthermore, by the intermolecular binding force, a DNA condensate is produced. This compaction is reversible with respect to the concentration of the fullerene derivative and the regeneration of the DNA takes place upon extractive removal of the fullerene derivative.

The foregoing has been confirmed by the agarose electrophoretic analysis and AFM microscopic morphological observation of samples prepared by

causing a varying concentration of the "tetramine compound", to be described below, to act upon the plasmid pBR322. This will be explained below using specific experimental data.

Test compound

The fullerene derivative obtained in Example 1 which appears below (hereinafter referred to as "tetramine compound") was submitted to the experiment.

Electrophoresis experiment protocol

Electrophoresis was carried out in accordance with the method described in Short Protocols in Molecular Biology 3rd E., 1992, Wiley, 2-13.

As test samples, solutions prepared by dissolving the plasmid pBR322 (25 μg/mL) and various amounts of "tetramine compound" in 20% THF/HEPES-Mg buffer (20 μL) were used. Each sample was incubated at 25°C for 5 minutes and then developed on an agarose gel using a buffer solution (5 μL) containing 0.25% (w/v) Bromophenol Blue and 50% (v/v) glycerol. Electrophoresis was carried out using an ethidium bromide (0.5 mg/mL)-containing 1% (w/v) agarose gel/TBE buffer solution. The integrated optical density (IOD) of the fluorescent emission photograph was measured

using NIH Image Program v1. 60. By this method, the migration amount of DNA was determined.

Results

The details are shown in Fig. 1.

Depending on the concentration of "tetramine compound", a phase transition phenomenon occurred. Thus, the amount of DNA migration in agarose electrophoresis declined rapidly when the ratio of the number of molecules of fullerene derivative to the number of base pairs of DNA was 1/1 and became nil at 1/2.6.

In the AFM microscopic observation of the same samples in a thin film of water, quite dissimilar AFM images were obtained before and after phase transition. Compaction of the DNA began before onset of phase transition and it was confirmed that the polymolecular compact obtained after phase transition was a hydrophobic mass.

The above experimental results suggest that in order that a wholesome DNA compact may be formed, the ratio of the number of molecules of the fullerene derivative to the number of base pairs of DNA preferably lies within the range of 4:1 to 1:2.

The above formation of a DNA condensate by the

fullerene derivative is carried out by mixing the two reactants in a suitable buffer. However, this is not an exclusive choice but any other method in routine use in the art can be employed. Moreover, the DNA condensate formed can be isolated by the routine procedure, for example by subjecting the DNA solution after phase transition to ethanol precipitation. Furthermore, by extracting the fullerene derivative from the solution of DNA which has been compacted by addition of the derivative with an organic solvent such as chloroform, regeneration of the original DNA can be achieved.

As mentioned hereinbefore, the DNA-compacting activity of the fullerene derivative is closely associated with its high binding affinity for DNA. For reference, results of a relevant experiment with said "tetramine compound" are shown below.

Experimental method

DNA-compacting activity was evaluated in an ethidium bromide displacement assay. This competitive binding assay was carried out according to the protocol described in Journal of Medicinal Chemistry, <u>21</u>, 658-668 (1978).

Results

The "tetramine compound" at a concentration of 1.9 μM substituted 50% of ethidium bromide.

This result can be used as a reference in the molecular designing of a fullerene derivative which is particularly useful for carrying the present invention into practice and a derivative having an equivalent or higher binding affinity for DNA as compared with the compound mentioned just above is especially desirable.

For exploring into the mechanism of DNA compaction by the fullerene derivative of the invention, the following two experiments were performed.

Experimental method

Measurement of the CD spectrum

A solution of calf thymus DNA (average MW = 8.6 MDa, 13 kbp, 42% GC, highly polymerized, type 1, Sigma) or plasmid pBR322 DNA (MW = 2.83 MDa, 4361 bp, New England Biolabs) in HEPES-Mg buffer (40 mM HEPES, 10 mM MgCl2; pH = 7.6) at a base-pair concentration of 100 mM was prepared and put in a quartz glass cell, and using a JASCO J-720 spectrometer the CD spectrum was measured at 25°C. Furthermore, the nucleic acid-compacting fullerene derivative was added to the above solution at concentrations 10 to 200 mM and the CD spectra were

measured.

Results

No change at all occurred in the DNA conformational CD spectra, indicating that the DNA retained the B-form.

Experimental method

Measurement of DNA melting temperature (Tm)

A solution of calf thymus DNA (average MW = 8.6 MDa, 13 kbp, 42% GC, highly polymerized, type 1, Sigma) in HEPES-Mg buffer (40 mM HEPES, 10 mM MgCl2; pH = 7.6) at a base-pair concentration of 100 mM was prepared and the nucleic acid-compacting fullerene was added at a concentration of 10 mM.

The above solution was put in a quartz glass cell and while the temperature was raised from 60°C at a rate of 1°C/min, the absorbance at the wavelength of 258 nm where the hypochromism of a DNA double-strand is observed, was measured with a JASCO J-720 spectrometer.

Results

The melting temperature of the double-strand was increased by 2.7°C to 74.2°C as compared with the case in which the nucleic acid-compacting fullerene was not

added (71.5°C), indicating stabilization of the double-stranded structure.

The above experimental results strongly suggested the mechanism that the DNA unimolecule is folded as the result of an interaction between the nucleic acid-compacting fullerene and the hydrophobic moiety of DNA (e.g. the major grooves of a DNA) and an interaction between the nitrogen-containing hydrophilic side chain and the phosphate group of the DNA, and that the hydrophobic moieties of a large number of folded DNA unimolecules coalesce to cause said compaction.

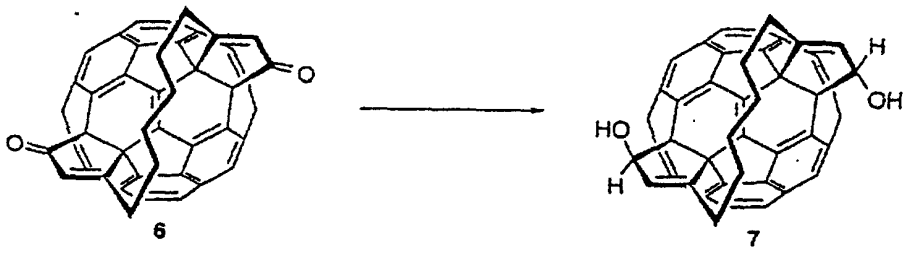
It is quite a novel finding that the fullerene derivative of the present invention not only has a binding affinity for DNA but even has an ability to compact a polymolecular DNA.

Therefore, "the mode of use for DNA compaction" and the mode of use as a "DNA compaction reagent" of the present invention include all possible embodiments exploiting the DNA-compacting activity of the fullerene derivative of the invention described hereinbefore. Included, among such embodiments, are the use as a DNA compaction reagent; use for introduction of a vector into cells; use for introduction of a DNA (or a derivative thereof) fragment such as an antisense DNA (or a derivative thereof) or a decoy DNA (or a derivative

thereof) into cells; use for control of gene expression through the binding to a promoter or enhancer region; use for modulating the cell cycle through suppression of the conversion of a double-stranded DNA to a single-stranded DNA; and use for control of the PCR efficiency through adjustment of the melting point involved in the transition from a single-stranded DNA to a double-stranded DNA or from a double-stranded DNA to a single-stranded DNA. Furthermore, application to gene therapies is also within the purview.

In using the fullerene derivative in any of the above modes of use, the fullerene derivative or salt of the invention can be used as it is or in the form of a composition to accomplish the intended object.

EXAMPLES



6 7

Preparation 1

To a solution of dienone 6 (130 mg, 143 μmol) in chlorobenzene (130 mL) was added diisobutylaluminum

hydride (in hexane) (0.95 M, 751 μL) slowly at room temperature. After 2 hours of constant stirring, a 30% aqueous solution of potassium sodium tartrate was added, and the mixture was stirred for 1 hour. The crude solution was washed with water, and removal of organic solvent in vacuo gave a crude product as a sparingly soluble black solid mass (130 mg). This diol 7 is a mixture of C_2 symmetric and C_1 symmetric diastereomers (about 7:3). This mixture is not further purified but submitted directly to the subsequent reaction.

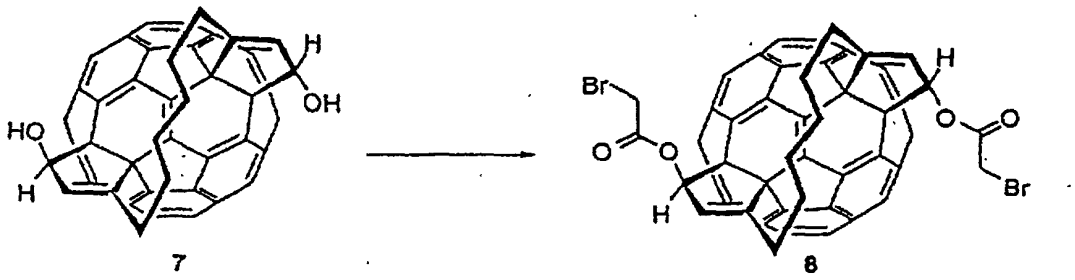
Diol 7

R_f=0.15 (PhCl)

IR (KBr): 3417, 2925, 1506, 798, 694 cm^{-1}

^1H NMR (400 MHz, $CDCl_3/CS_2$1/1) δ 1.54-1.62 (br m, 4H, $(CH_2)_2$-$(CH_2)_2$-$(CH_2)_2$), 1.76-1.88 (br m, 2H, homoallylic methylene proton), 1.88-1.99 (br m, 2H, homoallylic methylene proton), 2.28 (d, 2H, J=12.0 Hz, OH), 2.65-2.80 (m, 4H, allylic methylene proton), 6.22 (br s, 2H, vinyl proton), 6.34 (br d, 2H, J=12.0 Hz, allylic methylene proton)

Preparation 2



To a solution of diol 7 (50 mg, 54.7 μmol) in chlorobenzene (50 mL) were added bromoacetyl bromide (23.7 μL, 274 μmol) and pyridine (22.1 μL, 274 μmol). After 6 hours of constant stirring, the reaction was stopped by adding sodium hydrogen carbonate. Extraction with water gave a crude product. Purification by silica gel column chromatography (silica gel 5 g, elution with chlorobenzene) gave dibromide 8 (31.6 mg, 50%, 2 steps).

Dibromide 8

R_f=0.65 (PhCl)

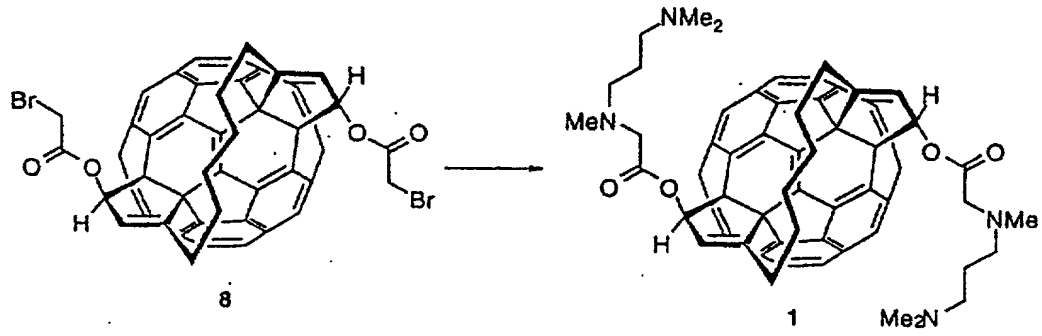
IR (KBr): 2925, 2854, 1733, 1261, 694 cm^{-1}

^1H NMR (400 MHz, $CDCl_3$) δ 1.55-1.63 (br m, 4H, $(CH_2)_2$-$(CH_2)_2$-$(CH_2)_2$), 1.80-2.00 (br m, 4H, homoallylic methylene proton), 2.70-2.84 (br m, 4H, allylic methylene proton), 3.80 (d, 2H, J=12.0 Hz, $BrCH_2CO$), 3.84 (d, 2H, J=12.0 Hz, $BrCH_2CO$), 6.16 (br s, 2H, vinyl proton), 7.33 (br s, 2H, allylic methylene proton)

^{13}C NMR (100 MHz, $CDCl_3$) δ 25.66 (CH_2), 27.03 (CH_2), 29.33 (CH_2), 73.11 (sp^3, C60), 74.11 (sp^3, C60), 88.04 (allylic

CH), 125.05 (vinyl CH), 127.24, 132.98, 136.38, 136.52, 138.30, 139.56, 141.79, 141.93, 142.07, 142.11, 143.14, 144.74, 144.76, 144.90, 145.32, 145.41, 145.62, 145.67, 146.01, 146.05, 146.35, 147.36, 147.61, 147.83, 148.34, 148.74, 148.98, 149.87, 152.51, 166.78 (C=O)

Example 1



To a solution of dibromide 8 (23.1 mg, 20.0 μmol) in chlorobenzene (10 mL) was added N,N,N'-trimethyl-1,3-propanediamine (14.7 μL, 100 μmol). After 1 hour of constant stirring, aqueous extraction was carried out to give a crude product. Purification by gel permeation chromatography (JAIGEL-1H 20×600 mm and -2H 20×600 mm GPC columns, elution with 0.5% triethylamine/chloroform) gave tetramine 1 (12.2 mg, 50%).

Tetramine 1

R_f=0.05 ($CHCl_3$/MeOH/AcOH 85/10/5)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40-1.54 (overlapped m, 8H,

NCH_2CH_2 and $(CH_2)_2$-$(CH_2)_2$-$(CH_2)_2$), 1.50-1.75 (br m, 2H, homoallylic methylene proton), 1.75-2.00 (br m, 2H, homoallylic methylene proton), 2.08 (overlapped s, 16H, $N(CH_3)_2$ and NCH_2), 2.20 (s, 6H, NCH_3), 2.36 (t, 4H, J=7.4 Hz, NCH_2), 2.60-2.74 (br m, 4H, allylic methylene proton), 3.15 (d, 2H, J=17.2 Hz, NCH_2CO), 3.28 (d, 2H, J=17.2 Hz, NCH_2CO), 6.08 (br s, 2H, vinyl proton), 7.35 (br s, 2H, allylic methylene proton)

^{13}C NMR (100 MHz, $CDCl_3$) δ 25.53 (CH_2), 25.96 (CH_2), 26.91 (CH_2), 29.29 (CH_2), 42.09 (NCH_3), 45.55 ($N(CH_3)_2$), 54.63 (CH_2), 57.55 (CH_2), 58.30 (CH_2), 73.28 (sp^3, C60), 74.08 (sp^3, C60), 86.68 (allylic CH), 125.86 (vinyl CH), 127.24, 132.76, 136.34, 136.40, 138.19, 139.49, 141.80, 141.83, 141.86, 142.08, 143.17, 144.65, 144.73, 144.97, 145.20, 145.44, 145.46, 145.65, 145.96, 145.99, 146.29, 147.52, 147.82, 148.71, 148.91, 148.96, 150.23, 151.21, 155.30, 170.68 (C=O)

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 shows the result of an experiment in which the "tetramine compound" was subjected to agarose gel electrophoresis. The ordinate represents IOD (%) and the abscissa represents the ratio of the number of molecules of the "tetramine compound" to the number of base pairs of DNA.

CLAIMS

1. A fullerene derivative having 1 to 4 nitrogen-containing hydrophilic side chain(s) or a salt thereof for use for DNA compaction.

2. The fullerene derivative or a salt thereof for use for DNA compaction as claimed in Claim 1, wherein the nitrogen-containing hydrophilic side chain is "a hydrocarbon group which has 1 or 2 straight-chain or branched-chain substituent group(s) each comprising 1 to 10 nitrogen atom(s) and 2 to 30 carbon atoms, and is configured to be bonded to 1 or 2 of the 2 to 8 sp^3 carbon atoms present on the fullerene core" (provided, however, that there may exist a cross-linking moiety comprising an alkylene group bridging two or more nitrogen-containing hydrophilic side chains).

3. The fullerene derivative or a salt thereof for use for DNA compaction as claimed in Claim 2, which has one or two nitrogen-containing hydrophilic side chains.

4. The fullerene derivative or a salt thereof for use for DNA compaction as claimed in Claim 3, wherein the nitrogen-containing hydrophilic side chain is "a

hydrocarbon group which has 1 or 2 straight-chain or branched-chain substituent group(s) each comprising 2 to 8 nitrogen atoms and 2 to 20 carbon atoms, and is configured to be bonded to two of the 2 to 8 sp^3 carbon atoms present on the fullerene core" (provided, however, that there may exist a cross-linking moiety comprising an alkylene group bridging two nitrogen-containing hydrophilic side chains).

5. Use of a fullerene derivative having 1 to 4 nitrogen-containing hydrophilic side chain(s) or a salt thereof for DNA compaction.

6. Use of the fullerene derivative or a salt thereof for DNA compaction as claimed in Claim 5, wherein the nitrogen-containing hydrophilic side chain is "a hydrocarbon group which has 1 or 2 straight-chain or branched-chain substituent group(s) each comprising 1 to 10 nitrogen atom(s) and 2 to 30 carbon atoms, and is configured to be bonded to 1 or 2 of the 2 to 8 sp^3 carbon atoms present on the fullerene core" (provided, however, that there may exist a cross-linking moiety comprising an alkylene group bridging two or more nitrogen-containing hydrophilic side chains).

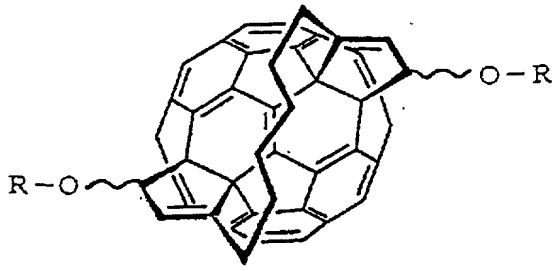
7. Use of the fullerene derivative or a salt thereof for DNA compaction as claimed in Claim 6, wherein said fullerene derivative or a salt thereof has 1 or 2 nitrogen-containing hydrophilic side chain(s).

8. Use of the fullerene derivative or a salt thereof for DNA compaction as claimed in Claim 7, wherein the nitrogen-containing hydrophilic side chain is a "a hydrocarbon group which has 1 or 2 straight-chain or branched-chain substituent group(s) each comprising 2 to 8 nitrogen atoms and 2 to 20 carbon atoms, and is configured to be bonded to two of the 2 to 8 sp^3 carbon atoms present on the fullerene core" (provided, however, that there may exist a cross-linking moiety comprising an alkylene group bridging two nitrogen-containing hydrophilic side chains).

9. A DNA compaction as formed by using a fullerene derivative having 1 to 4 nitrogen-containing hydrophilic side chain(s) or a salt thereof.

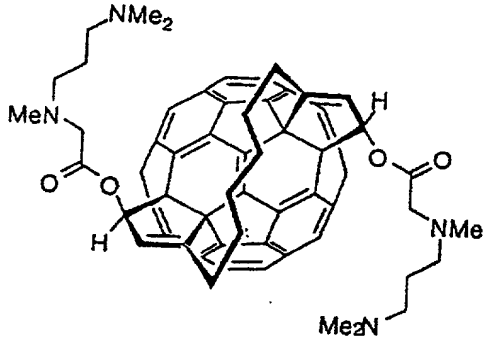
10. A DNA compaction as claimed in Claim 9, wherein the ratio of the number of molecules of the fullerene derivative or a salt thereof to the number of base pairs of the DNA is 4:1 to 1:2.

11. A fullerene derivative of the following general formula or a salt thereof:



[wherein the two Rs may be the same or different and each represents a straight-chain or branched-chain acyl group comprising 1 to 10 nitrogen atom(s) and 2 to 30 carbon atoms or hydrogen (provided, however, that the two Rs do not concurrently represent hydrogen)];

exclusive of the fullerene derivative of the following formula:

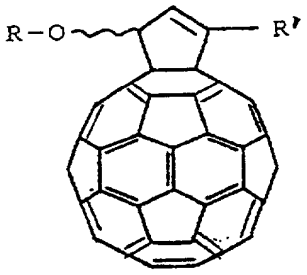


12. A fullerene derivative or a salt thereof as claimed in Claim 11, wherein the two Rs are the same or different and each represents a straight-chain or branched-chain acyl group comprising 2 to 8 nitrogen

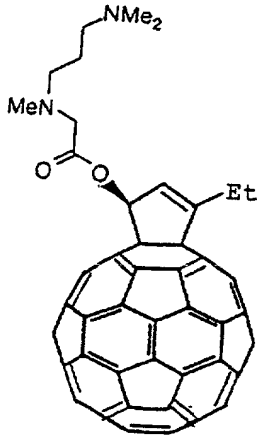
atoms and 2 to 20 carbon atoms.

13. A fullerene derivative or a salt thereof as claimed in Claim 12, wherein the two Rs are the same or different and each represents a [N-(N,N-di(lower)alkylamino)(lower)alkyl-N-(lower)alkyl]amino(lower)alkanoyl group.

14. A fullerene derivative of the following general formula or a salt thereof:



[wherein R represents a straight-chain or branched-chain acyl group comprising 1 to 10 nitrogen atom(s) and 2 to 30 carbon atoms and R' represents hydrogen or a lower alkyl group]; exclusive of the fullerene derivative of the following formula:



15. A fullerene derivative or a salt thereof as claimed in Claim 14, wherein R represents a straight-chain or branched-chain acyl group comprising 2 to 8 nitrogen atoms and 2 to 20 carbon atoms.

16. A fullerene derivative or a salt thereof as claimed in Claim 15, wherein R is an [N-(N,N-di(lower)alkylamino)(lower)alkyl-N-(lower)alkyl]amino(lower)alkanoyl group.

17. A DNA compaction reagent comprising a fullerene derivative having 1 to 4 nitrogen-containing hydrophilic side chain(s) or a salt thereof.

18. A DNA compaction reagent comprising a fullerene derivative or a salt thereof as claimed in Claim 17, wherein the nitrogen-containing hydrophilic side chain is "a hydrocarbon group which has 1 or 2 straight-chain or branched-chain substituent group(s) each comprising 1 to 10 nitrogen atom(s) and 2 to 30 carbon atoms, and is configured to be bonded to 1 or 2 of the 2 to 8 sp^3 carbon atoms present on the fullerene core" (provided, however, that there may exist a cross-linking moiety comprising an alkylene group bridging two or more nitrogen-containing hydrophilic side chains).

19. A DNA compaction reagent comprising a fullerene derivative or a salt thereof as claimed in Claim 18, which has 1 or 2 nitrogen-containing hydrophilic side chain(s).

20. A DNA compaction reagent comprising a fullerene derivative or a salt thereof as claimed in Claim 19 wherein the nitrogen-containing hydrophilic side chain is "a hydrocarbon group which has 1 or 2 straight-chain or branched-chain substituent group(s) each comprising 2 to 8 nitrogen atoms and 2 to 20 carbon atoms, and is configured to be bonded to two of the 2 to 8 sp^3 carbon atoms present on the fullerene core" (provided, however, that there may exist a cross-linking moiety comprising an alkylene group bridging two nitrogen-containing hydrophilic side chains).

21. Use of a fullerene derivative having 1 to 4 nitrogen-containing hydrophilic side chain(s) or a salt thereof for the manufacture of a DNA compaction reagent.

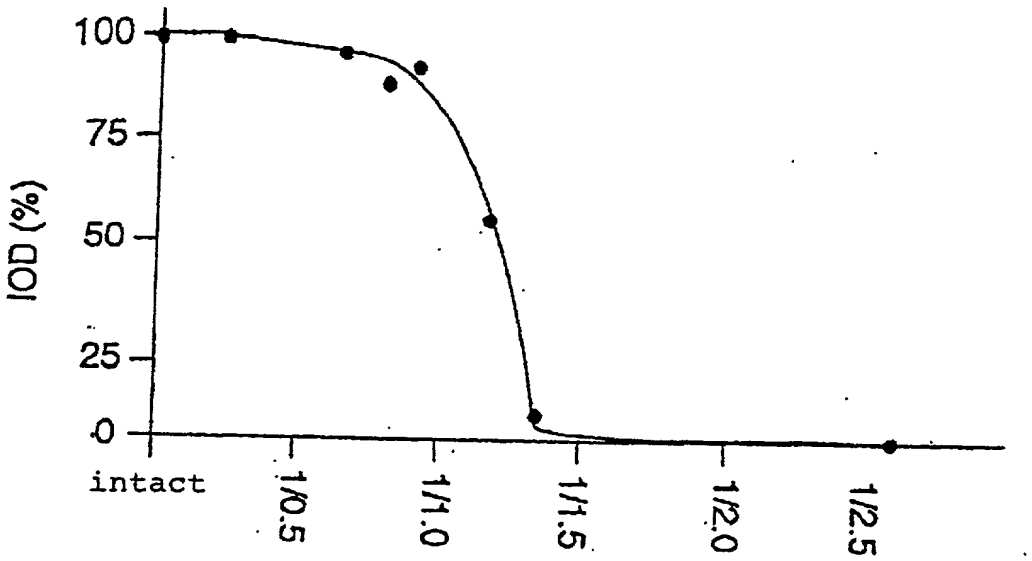
ABSTRACT

A novel means for DNA compaction is provided.

The object is accomplished by providing a fullerene derivative having 1 to 4 nitrogen-containing hydrophilic side chain(s) or its salt for DNA compaction.

DNA compaction can be achieved effectively, and application to gene therapy is also expected.

Fig.1



number of base pairs of DNA/number of molecules of "tetramine compound"

Japanese Language Declaration
（日本語宣言書）

委任状：私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。
（弁護士、または代理人の指名及び登録番号を明記のこと）

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

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第二の共同発明者の署名　日付	Second joint Inventor's signature　Date Masaya Sawamura　Oct. 25, 2000
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（第三以降の共同発明者についても同様に記載し、署名すること）

(Supply similar information and signature for third and subsequent joint inventors.)

Japanese Language Declaration
（日本語宣言書）

第三の共同発明者の氏名	Full name of third joint inventor, if any Hiroyuki ISOBE
第三の共同発明者の署名　日付	Third joint Inventor's signature　Date Oct.26, 2000
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第四の共同発明者の氏名	Full name of fourth joint inventor, if any
第四の共同発明者の署名　日付	Fourth joint Inventor's signature　Date
住所	Residence
国籍	Citizenship
郵便の宛先	Post Office Address

第五の共同発明者の氏名	Full name of fifth joint inventor, if any
第五の共同発明者の署名　日付	Fifth joint Inventor's signature　Date
住所	Residence
国籍	Citizenship
郵便の宛先	Post Office Address

第六の共同発明者の氏名	Full name of sixth joint inventor, if any
第六の共同発明者の署名　日付	Sixth joint Inventor's signature　Date
住所	Residence
国籍	Citizenship
郵便の宛先	Post Office Address

（第六またはそれ以降の共同発明者に対しても同様な情報および署名を提供すること。）

(Supply similar information and signature for third and subsequent joint inventors.)

Declaration and Power of Attorney For Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者（下記の名称が複数の場合）であると信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

FULLERENE DERIVATIVE S

上記発明の明細書は、

the specification of which

☐ 本書に添付されています。

☐ is attached hereto.

☒ ___月___日に提出され、米国出願番号または特許協定条約国際出願番号を_____とし、
（該当する場合）_____に訂正されました。

☒ was filed on September 7, 2000
as United States Application Number or
PCT International Application Number
09/622,915 and was amended on
_____ (if applicable).

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第37編第1条56項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

Japanese Language Declaration
(日本語宣言書)

私は、米国法典第35編119条（a）-（d）項又は365条（b）項に基づき下記の、米国以外の国の少なくとも一ヵ国を指定している特許協力条約365（a）項に基づく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

I hereby claim foreign priority under Title 35, United States Code, Section 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)
外国での先行出願

(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	Priority Claimed 優先権主張 Yes はい	No いいえ
10/58614	JAPAN	10 March 1998	☒ XX	☐
			☐	☐

私は、第35編米国法典119条（e）項に基づいて下記の米国特許出願規定に記載された権利をここに主張いたします。

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)

私は、下記の米国法典第35編120条に基づいて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約365条（c）に基づく権利をここに主張します。また、本出願の各請求範囲の内容が米国法典第35編112条第1項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日以降で本出願書の日本国内または特許協力条約国際提出日までの期間中に入手された、連邦規則法典第37編1条56項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of application.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)
PCT/JP99/01146	10 March 1999	

私は、私自信の知識に基づいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じるところに基づく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.